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GERMINATION INHIBITION IN SEED OF *CEANOTHUS PROSTRATUS* BENTH.[1]

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ABSTRACT

Studies revealed that seed dormancy in squaw carpet (Ceanothus prostratus) *could be due to a chemical inhibitor and to physiologically immature embryos. Isolation of chemical compounds in the developing seed was done by paper and gas chromatography. Inhibition was tested by germination assay. Metabolism in the after-ripening seed was indicated by changes in the lipid ratios in the seed. Successful germination is dependent on removal or destruction of the inhibiting material by a pretreatment consisting of either boiling for 10 minutes in water or soaking in acetone for 5 minutes, followed by after-ripening at 10° C. for 60 or more days.*

Land denuded of vegetation by natural disaster or disturbed by the activities of man is subject to accelerated erosion. It is desirable, therefore, to rapidly establish a plant cover to minimize erosional damage. The ideal cover would be relatively low growing and spreading but not to the extent of excluding other species. Squaw carpet *(Ceanothus prostratus)*, a native of the Sierra Nevada, offers such possibilities. However, for purposes of economy, a plant must establish itself readily from seed (Brown and others 1971). Without treatment, germination of squaw carpet is slow and generally less than 5 percent; thus, treatment of the seed may be required.

Treating the seed with acetone for 5 minutes followed by rinsing in cold water, or boiling in water for 10 minutes and allowing to steep until cool, increased the germination to 24 and 16 percent, respectively. Subjecting the seed to after-ripening at 0° C.

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and 10° C. for periods of 60 or more days without pre-treatment did not result in significant increases over the control. Pre-treatment followed by after-ripening at only 0° C. was not effective in increasing germination above that for pre-treatment alone. However, germination was increased when pre-treatment was followed by after-ripening at 10° C. for periods of 60 or more days. Sixty-five percent germination was obtained by boiling the seed and then holding them for 68 days of after-ripening at 10° C.

The germination obtained with these pre-treatments strongly indicated the presence within the seed of some substance that blocks metabolism. Removal or destruction of this material is necessary before the after-ripening process can occur.

MATERIALS AND METHODS

Each treatment required 10 grams of *C. prostratus* seed collected in the Sierra Nevada for this study. The test seed was boiled and soaked in acetone in the light and then subjected to varying periods of after-ripening at 10° C. and compared to the control which received no treatment and was stored dry at room temperature.

Metabolic changes were studied by analysis of the lipid fraction obtained from periodic extraction of the seed samples. The method of Mirocha and DeVay (1961) was used to esterify the fatty acids before analysis by gas chromatography. Changes in the proportions of the fatty acids were considered to be an indication of metabolism.

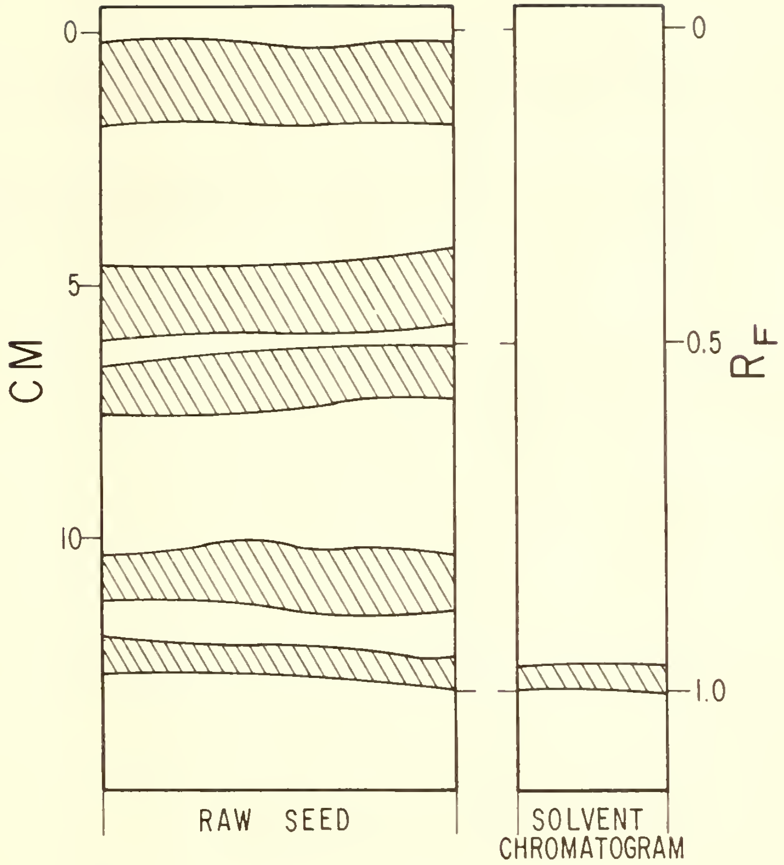
The water used for boiling, and the acetone used to soak the seed, were examined by paper and gas chromatography to determine if any material had been removed from the seed during treatment.

The method outlined by Hilton and others (1965) was used to prepare and chromatograph the seed extract. The seed capsule of *C. prostratus* normally contains three seeds which are forcefully ejected when mature. This factor contributes to the difficulty of harvesting large quantities of seed. Untreated *C. prostratus* seed germinates irregularly over a period of several weeks and at a rate of less than 5 percent. Therefore, for the bioassay, seed of the common tomato *(Lycopersicon escutentum)* was selected as a dependable responsive organism to test the effect of any substance found on the chromatograms. These seeds were germinated individually on 1-cm.^{2} pieces of filter paper having the following characteristics: control (no treatment); blank chromatogram (solvent system); and chromatograms of raw seed extract (aqueous suspension of macerated untreated seed). The pieces of filter paper were placed in individual cells of miniature polyethylene ice-cube trays. After placement of the tomato seed, the filter paper was moistened and the trays were wrapped in a sheet of household Saran. The seed was held at room temperature and the percent of germination recorded at 12-hour intervals for a total period of 156 hours.

RESULTS

When observed under longwave ultraviolet light, five fluorescent bands were detected in the untreated seed extract that included the solvent front (fig. 1). Only the solvent front was detectable on the blank (solvent) chromatogram (fig. 1). The bioassay revealed germination inhibition at the solvent front of both chromatograms, indicating the presence of some substance in the filter paper that was concentrated by the solvent and inhibitory to the germination of tomato seed. Inhibition was also found in two bands of the raw seed extract. A band located at approximately R_f 0.7 had a transitory effect on germination. The band located at approximately R_f 0.9 was inhibitory throughout the entire period of testing (fig. 2). The analysis of variance of the 156-hour data reveals a treatment difference that is significant at the 10-percent level of probability. Therefore, it is probable that the R_f 0.9 band contained an unknown germination inhibitor that was active on tomato seed. The molecular weight of this inhibiting substance at band R_f 0.9 was not determined. However, the substance characteristically increases the solution vapor pressure when dissolved in absolute ethanol. Therefore, it is one of relatively few compounds.

Figure 1.--Chromatograms of raw seed extract and solvent system. White fluorescence is indicated by the shaded bands.



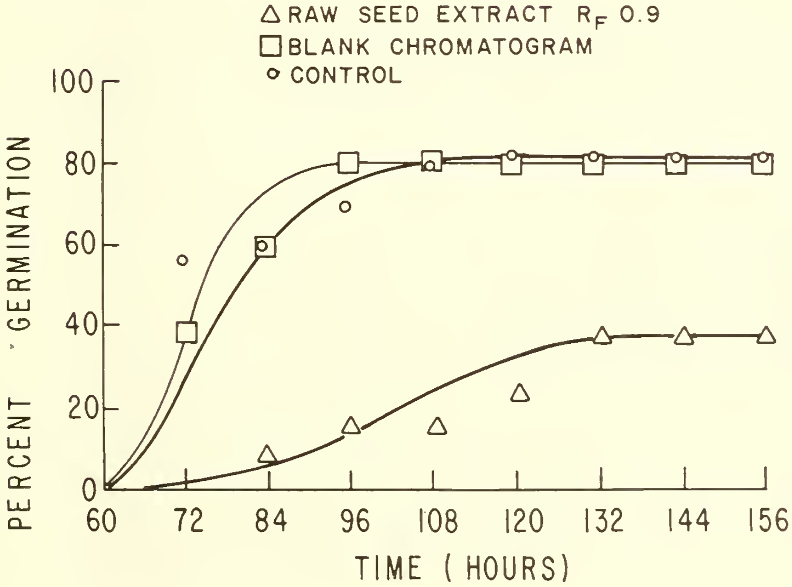


Figure 2.--The effect of band R_f 0.9 on the germination of tomato seed.

Table 1.--Effect of boiling and after-ripening on the comparative amounts of fatty acids in the seed of C. prostratus

Treatment	Fatty acids			
	Palmitic	Oleic	Linoleic	Linolenic
	Percent			
Raw seed	40	81	100	60
Boiled	48	82	100	52
Boiled plus 7 days at 10° C.	54	79	100	32
Boiled plus 14 days at 10° C.	54	71	100	29

Detectable changes in the amounts of fatty acids in the seed occurred during the after-ripening process when this process was preceded by boiling (tables 1 and 2).

No lipids were detectable in the water that had been used for boiling or in the acetone used for soaking the seed. Both the water and the acetone were chromatographed by a procedure identical to that employed for the seed extract. Furthermore, no material except the solvent front was found in the water when examined under ultraviolet light, and this could not be distinguished from the solvent front of the blank chromatogram. The solvent front of the chromatogram prepared from the acetone was somewhat wider and more intense than that of the blank chromatogram.

Table 2.--A comparison of the utilization of fatty acids during after-ripening of C. prostratus *seed*

Treatment	Fatty acids			
	Palmitic	Oleic	Linoleic	Linolenic
	Percent			
Raw seed	100	100	100	100
Boiled	130	112	105	96
Boiled plus 7 days at 10° C.	88	65	67	36
Boiled plus 14 days at 10° C.	76	50	57	28

DISCUSSION

Previous studies showed that successful germination of squaw carpet is dependent on treatment of the seed and a period of after-ripening at a temperature that permits occurrence of metabolic processes. Bioassay of the raw seed extract did show at R_f 0.9 the presence of a material that is inhibitory to the germination of tomato seed. Although there is no direct evidence that this material is inhibitory to *C. prostratus*, the changes in the total amounts of fatty acids and their ratios to the amounts of linoleic acid after treatment indicate that the extract could be involved in the metabolism of this species. Destruction or removal of this inhibiting material is necessary before the metabolic changes that result in germination can take place during the after-ripening process. The dormancy of this species appears, then, to be attributable to two factors: the presence of an inhibitor; and physiologically immature embryos (Amen 1964).

Wareing and Foda (1956) reported the occurrence of fluorescent inhibitors in *Xanthium* and *Lactuca* seed; also, it was noted that these inhibitors lost their fluorescent properties when subjected to high oxygen tension in the presence of a cell-free extract of the seed. The reduction or modification of the lipid content of the testa would allow a greater exchange of gases to take place between the embryo and the environment; however, since the analysis of the agents used was negative for lipids, such an exchange would seem unlikely in the present study. Chromatographic studies of the seed extracts before and after treatment indicate a different mechanism for the two treatments used in this study. Band R_f 0.9 is not detectable on chromatograms made immediately following treatment with boiling water or acetone (fig. 3); this indicates destruction or removal of this band by the two agents without the involvement of an enzyme-controlled system.

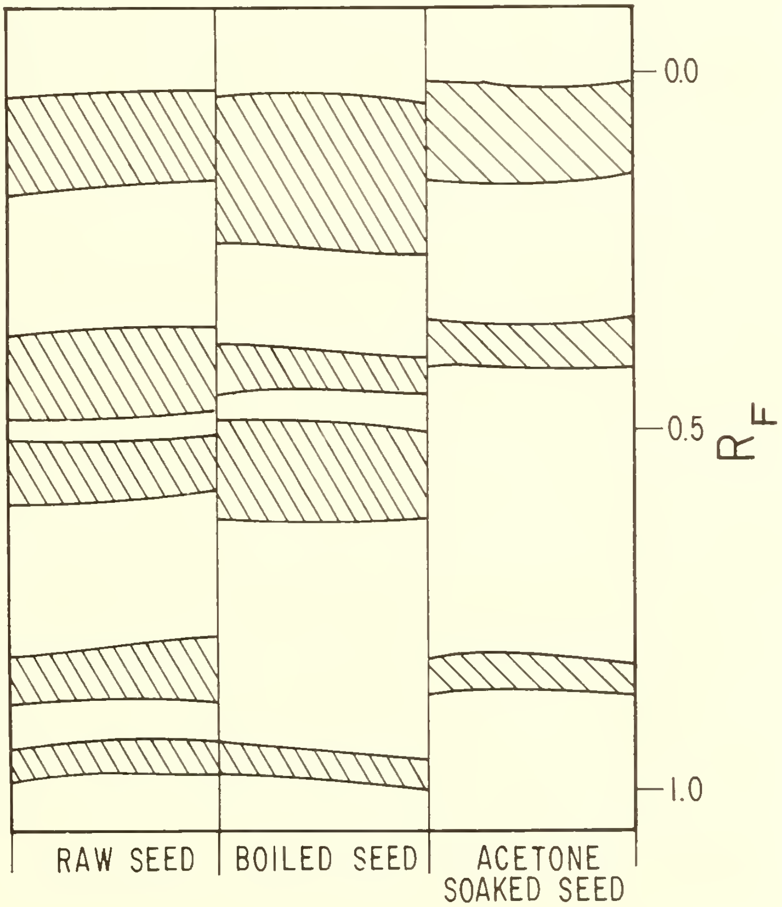


Figure 3.--Chromatograms showing the effect of boiling and acetone soak on detectable fluorescent bands in C. prostratus *seed.*

Chromatography of the water used to boil the seed was negative for any fluorescent material other than the solvent front; thus, boiling the seed in water must be responsible for the destruction of the material in band R_f 0.9. This destruction could be a direct action such as hydrolysis of the substance. A report by Nutile (1943), however, indicates that boiling water might be only indirectly involved in the destruction of the inhibitor. For example, he was unable to induce dormancy in lettuce seed with coumarin if the seed was exposed to light while still wet. Furthermore, since all the treatments in this study were carried out in the light, there is a possibility that the boiling treatment only serves to moisten the seed sufficiently to allow light, as a direct agent, to destroy the fluorescent and inhibitory properties of the material of band R_f 0.9.

The mechanism of acetone, on the other hand, differs from that of boiling water. The acetone treatment removes the materials that appear as fluorescent bands at R_f 0.7 and R_f 0.9 (fig. 3). The chromatogram of the acetone used for treatment does show an increase in the intensity of fluorescence at the solvent front in comparison with the blank chromatogram, but there is a difference in the position of the material. Milborrow (1963) suggested that acetone could be used to remove inhibitory materials from seed. In this study, the difference in position on the chromatogram could indicate that removal might be accompanied by simultaneous modification of the inhibitory substance.

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